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(54) Title: ENHANCEMENT OF THE DOWN-REGULATION OF AUTOIMMUNE DISEASES BY ORAL ADMINISTRATION OF AUTOANTIGENS (57) Abstract Disclosed herein are methods to prevent or treat T-cell dependent autoimmune diseases comprising the oral administration of (A) an agent selected from the group consisting of autoantigens, fragments or analogs thereof specific for the autoimmune disease, and (B) a synergist having the property of enhancing the autoimmune disease suppressive activity of the agent, in amounts effective to treat or prevent a T-cell dependent autoimmune disease. Also disclosed are pharmaceutical formulations or dosage forms for use in the methods.		

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ENHANCEMENT OF THE
DOWN-REGULATION OF AUTOIMMUNE DISEASES BY
ORAL ADMINISTRATION OF AUTOANTIGENS

5 BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to an improvement in the treatment of autoimmune diseases and in particular T-cell-mediated or T-cell-dependent autoimmune diseases. More specifically, the present invention is directed to the use of synergists to augment the activity of orally or enterally administered autoantigens, or disease-suppressive fragments or analogs thereof, in the prophylactic and therapeutic treatment of autoimmune diseases.

15 Brief Description of the Background and Prior Art

Autoimmune diseases are characterized by an abnormal immune response (involving either cells or antibodies) directed against normal autologous tissues.

A number of strategies have been used or proposed to suppress autoimmune diseases, most notably drugs, such as clyclophosphamide, cyclosporin A, methotrexate, and Imuran (azothioprine). Steroid compounds, such as prednisone and methylprednisolone are also employed in many instances. These drugs have limited long term efficacy against both cell- and antibody-mediated autoimmune diseases. Use of such drugs is limited by virtue of their toxic side effects to other organ systems and also because they induce "global" immunosuppression

in a patient receiving prolonged treatment with these drugs, e.g. the normal protective immune response to pathogenic microorganisms is downregulated thereby increasing the risk of infections caused by these pathogens. A further drawback is that there is an increased risk that malignancies will develop in patients receiving prolonged global immunosuppression.

Other therapies have been proposed for the treatment of autoimmune diseases. U.S. Patent Application Serial No. 65,794 filed June 24, 1987 (now abandoned) and copending International Patent Application PCT/US88/02139 filed June 24, 1988, disclose that the oral or enteral administration of myelin basic protein (MBP) and disease-inducing and non-inducing fragments and analogs thereof is effective in suppressing acute monophasic experimental allergic encephalomyelitis (EAE), an induced T-cell mediated autoimmune disease directed against myelin basic protein (MBP). EAE is a recognized and widely used animal model for the human disease multiple sclerosis (MS). The above-identified applications also disclose that the oral or enteral administration of Mycobacterium tuberculosis is an effective treatment for suppressing adjuvant arthritis and extrapolate the aforementioned results to the treatment of other autoimmune diseases.

Copending U.S. Patent Application Serial No. 379,778 filed July 14, 1989 discloses the oral or enteral administration of S-antigen for the treatment of autoimmune uveoretinitis.

Copending U.S. Patent Application Serial No. 454,806 filed December 20, 1989 discloses the aerosol administration of autoantigens for the treatment of cell-and antibody-mediated autoimmune diseases.

Nagler-Anderson et al. (Proc. Natl. Acad. Sci. (USA) 83: 7443-7446, 1986) describe the oral administration of collagen to suppress collagen-induced arthritis in a mouse model.

Various methods have been employed to induce antigen-specific suppression of EAE such as immunization with MBP emulsified in incomplete Freund's adjuvant, (Lando, Z. et al.,

J. Immunol. 126: 1526 (1981)), and intravenous injection of MBP-conjugated lymphoid cells (Sriram, S. et al., Cell. Immunol. 75: 378 (1983)).

5 Traugott et al., J. Neurol. Sci. 56:65-73 (1982), and Raine et al., Lab. Investigation 48: 275-84 (1983) disclose that treatment of a strain of guinea pigs suffering from chronic relapsing EAE by parenteral administration of MBP alone or in incomplete Freund's adjuvant (IFA) or in combination with galactocerebroside, a lipid hapten of myelin, suppressed the
10 clinical EAE symptoms.

McKenna et al., Cell. Immun. 81: 391-402 (1983), disclose that preinjection of rats with guinea pig MBP coupled to syngeneic spleen leukocytes or to syngeneic red blood cells suppressed (in a dose-dependent manner) the subsequent induc-
15 tion of EAE using guinea pig MBP in Freund's complete adjuvant.

Based on an English language abstract, Belik et al., Vopr. Med. Khim. 24: 372-377 (1978), disclose (according to an English language abstract) parenteral administration of "alkaline myelin protein fragment" (AMPF) and "synthetic en-
20 cephalitogenic peptide" (SEP) to guinea pigs with EAE. The animals had been sensitized by bovine AMPF or by SEP but recovered after AMPF administration.

Braley-Mullen et al., Cell. Immun. 51: 408 (1980), and Nagler-Anderson et al. noted above, both disclose the suppres-
25 sion of the symptoms of two other experimental autoimmune diseases which are induced by injection of animals with autoantigen-lymphocyte conjugates. Braley-Mullen et al. reports the suppression of experimental autoimmune thyroiditis in the guinea pig by injection of thyroglobulin antigen in IFA. Nagler-Anderson et al. reports the suppression of Type II
30 collagen-induced arthritis in the mouse by intragastric administration of soluble, but not denatured, Type II collagens prior to immunization of the animal with Type II collagen in adjuvant.

35 Newby, T.J. et al. (Local Immune Responses in the Gut, p. 1191, CRC Press, Boca Raton, FL, 1984; Immunol. 41: 617-621, 1980) report that feeding bacterial LPS to mice can enhance

tolerance to contact-hypersensitivity induced by orally administered antigen (picryl chloride). However, simultaneous oral administration of LPS and picryl chloride had no statistically significant effect in delayed-type hypersensitivity (DTH) reactions to this antigen.

Michalek et al. (J. Immunol. 128: 1992-1998, 1982) report that prolonged gastric intubation (i.e. enteral administration) of sheep red blood cells (SRBC) followed by systemic administration of SRBC resulted in enhanced splenic immune responses in LPS-unresponsive mice and oral tolerance in LPS-responsive mice. There was no coadministration of LPS.

Mowat, A.M. et al. Immunol. 58: 677-683, 1986) disclose that simultaneous administration of LPS (intravenously) to normal mice fed ovalbumin prevented the induction of oral tolerance but did not alter the Delayed-type hypersensitivity (DTH) responses to fed ovalbumin.

Hamada, T. et al. (Autoimmunity 2: 275-284, 1989) report that LPS treatment in vitro of MBP-sensitized lymphoid cells augmented the adoptive transfer of EAE in Lewis Rats.

Due to the progressive, debilitating nature of autoimmune diseases, what is needed in the art are improved methods for treating autoimmune diseases.

It is an object of the present invention to provide improved methods for treating mammals suffering from autoimmune diseases.

It is a further object of the present invention to provide pharmaceutical formulations for treating mammals suffering from autoimmune diseases.

SUMMARY OF THE INVENTION

The present invention is directed to an improved method for treating or preventing a T-cell mediated or T-cell dependent autoimmune disease (AD) which comprises:

(a) orally or enterally administering to a mammal in need of such treatment at least one AD-suppressive agent selected from the group consisting of (i) autoantigens specific for said disease; (ii) AD-suppressive fragments of said

autoantigens; and (iii) AD-suppressive analogs of said autoantigens or said fragments; and

(b) also administering to said mammal at least one compound having the property of enhancing the AD-suppressive activity of said agent.

The amount of the enhancing compound should be effective to potentiate the AD-suppressive agent and effective to enhance the suppressive activity of the AD-suppressant if it is administered at a level already effective to suppress AD symptoms.

The invention also embraces synergist compositions and dosage forms useful in the treatment of AD.

The activity-enhancing compound (which will be often referred to herein as a "synergist") need not be administered orally or enterally; however, oral or enteral administration is preferred. Furthermore, any order of administration of the suppressive agent and the synergist is within the scope of the invention although substantially simultaneous administration is preferred.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a bar graph showing the effect of feeding MBP-LPS on delayed-type hypersensitivity (DTH) reactions in rats.

Figure 2 is a bar graph showing the effect of feeding MBP-Lipid A or DTH responses in rats.

Figure 3 (A-C) are a series of bar graphs showing the effect of feeding MBP + LPS on chronic EAE in guinea pigs.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

All patents, patent applications and literature references cited herein are hereby incorporated by reference in their entirety.

The present invention relates to the treatment of T-cell-mediated or T-cell-dependent autoimmune disease by (a) the oral or enteral administration of an AD-suppressive agent and (b) administration of one or more compounds that enhance the AD-suppressive activity of the agent.

As used herein, the term "treatment" is meant to include both prophylactic treatment to prevent autoimmune diseases (or the manifestation of clinical symptoms thereof) as well as the therapeutic suppression or alleviation of symptoms after the onset of such autoimmune diseases.

The term "mammal" covers all life forms that have an immunoregulatory system and are therefore susceptible to autoimmune diseases.

An autoimmune disease is a malfunction of the immune system of mammals, including humans, in which the immune system fails to distinguish between foreign substances within the mammal and autologous tissues or substances and, as a result, treats autologous tissues and substances as if they were foreign.

An "autoantigen" is any substance normally found within a mammal that, in an abnormal situation, is no longer recognized as part of the mammal itself by the lymphocytes or antibodies of that mammal, and is therefore attacked by the immunoregulatory system as though it were a foreign substance. The term also includes antigenic substances which induce conditions having the symptoms of an autoimmune disease when administered to mammals.

The term "AD-suppressive fragment(s)" of such autoantigens includes any peptide or polypeptide or other compound comprising partial amino acid sequences or moieties of autoantigens and possessing the ability to suppress or prevent T-cell-mediated or T-cell-dependent autoimmune response, upon oral or enteral administration. It should be noted that such fragment need not also possess the autoantigenic properties of the entire autoantigen. For example, with respect to MBP, which when administered parenterally with an adjuvant induces EAE in susceptible mammals, it was discovered that certain non-disease-inducing fragments of MBP nevertheless possessed AD-suppressive activity as disclosed in U.S. Patent Application Serial No. 65,794 and 454,806.

The term "analog(s)" of such autoantigens or fragments includes compounds that are so structurally related to these

autoantigens or to their AD-suppressive fragments that they possess the same biological activity, i.e., the ability to eliminate or suppress T-cell-mediated or T-cell-dependent autoimmune response, upon oral or enteral administration. For example, the term includes peptides having amino acid sequences which differ from the amino acid sequence of the autoantigen by one or more amino acid residues (while still retaining the AD-suppressive activity) as well as compounds or compositions which mimic the AD-suppressive activity of the autoantigens in their ability to suppress or alleviate the symptoms of the disease. An example of such compositions is tissue from an organ that is the target of attack in an autoimmune disease.

"Autoimmune-disease suppressive agent" or "AD-suppressive agent" is a compound or composition which when administered orally or enterally to a mammal suppresses, prevents or delays the clinical manifestation of a particular autoimmune disease. The term includes autoantigens specific for an autoimmune disease, as well as AD-suppressive fragments or analogs thereof as defined above.

"Synergists" are defined herein as substances which augment or enhance the suppression of the clinical manifestation of autoimmune diseases when administered orally in conjunction with administration of AD-suppressive agents. As used in the preceding sentence "in conjunction with" (also referred to herein as "in association with") means before, substantially simultaneously with or after oral or enteral administration of the AD-suppressive agent. Naturally, administration of the synergist should not precede nor follow that of the AD-suppressive agent by so long an interval of time that the effects of the substance administered first have worn off. Therefore, the synergists of the present invention should be administered within 24 hours of the AD-suppressive agent.

Examples of synergists for use in the present invention include, but are not limited to bacterial lipopolysaccharides from a wide variety of gram-negative bacteria such as various subtypes of E. coli or Salmonella (LPS, Sigma Chemical Co. St. Louis, MO, Difco, Detroit, MI BIOMOL Res. Labs, Plymouth

Meeting, PA), Lipid A (Sigma Chemical Co., ICN Biochemicals, Cleveland, OH, Polysciences Inc., Warrington, PA), and immunoregulatory lipoproteins, such as peptides covalently linked to tripalmitoyl-S-glycerylcysteinyl-seryl-serine (P₃CSS), which
5 can be obtained as disclosed in Deres, K. et al. Nature 342: 561-564, 1989) or "Braun's", lipoprotein from E. coli which can be obtained as disclosed in Braun, V., Biochim. Biophys. Acta 435: 335-337, 1976. LPS is preferred and Lipid A is particularly preferred. Lipid A is particularly preferred for use
10 in the present invention because it is less toxic than the entire LPS molecule. LPS for use in the present invention can also be extracted from gram-negative bacteria and purified using the methods of Galanes, C. et al. (Eur J. Biochem. 9: 245, 1969) and Skelly, R. R. et al. (Infect. Immun. 23: 287,
15 1979).

One common feature of the biologically active materials which can be used as synergists in the present invention is that they induce the production and/or secretion of interleukin-1 (IL-1).

20 IL-1 is a protein produced by many normal and transformed cells, and is predominantly produced by macrophages, monocytes and keritanocytes. Microbial products, such as endotoxina, exotoins, yeast cell walls, and viral hemagglutinins induce IL-1 production by monocytes. IL-1 has a broad spectrum
25 of biological activities on cells of the immune system, as well as cells outside the immune system, and is considered to be a major inflammatory mediator.

Administration of synergists in association with oral or enteral administration of an AD-suppressive agent markedly
30 enhances the activity of the agent in suppressing the symptoms of the autoimmune disease for which the agent is specific. In other words, the efficacy of treatment of the target autoimmune disease is increased by co-administration of the synergist (compared the efficacy of use of the AD-suppressant agent
35 alone).

According to the present invention, after an AD-suppressive agent is administered orally or enterally (i.e.

directly into the stomach) it passes into the small intestine where it comes in contact with Peyer's Patches cells which are collections of immunocytes located under the intestinal wall. These cells as well as intraepithelial lymphocytes (located
5 under the intestinal epithelium) are in communication with the immune system including the spleen and lymph nodes. The result is that tolerance to the AD-suppressive agent is induced and the immune response directed against the mammal's own tissues is attenuated or entirely suppressed. The thus-induced
10 tolerance is specific for the autoimmune disease: no effect has been discerned on the ability of the treated mammal to mount a response against a pathogen. Moreover, oral or enteral administration of an irrelevant antigen (i.e. one not implicated in an autoimmune disease, such as bovine serum albumin)
15 produced no effect on the clinical manifestation of the autoimmune disease or on the susceptibility of a mammal to an inducible condition (such as EAE or AA) serving as a model for an autoimmune disease.

The tolerance induced by the AD-suppressive agents of
20 this invention is dose-dependent: over a broad oral or enteral dosage range, the suppression (or attenuation) of clinical and histological manifestations of the disease increases with increasing dosage of AD-suppressive agent ingested.

The ability of the synergist to enhance orally-induced
25 tolerance by an autoantigen is also dose-dependent, i.e. it increases with increasing dose of synergist. But because the enhancement by the synergist of orally induced tolerance is indeed synergistic, use of the synergist is anticipated to continue to improve tolerance induced by relatively high oral
30 or enteral doses of the AD-suppressive agents. Conversely, associated use of the synergist is anticipated to permit lower doses of AD-suppressive agent to be used for the same level of clinical symptom suppression to be achieved.

The activity of the synergist in enhancing orally-
35 induced tolerance is not specific to the particular AD-suppressive agent. Both EAE and DTH responses were down-regulated upon co-administration of the specific autoantigen and the

synergists of the present invention. Thus, the ability of the synergist to enhance orally-induced specific tolerance to an autoantigen can be extrapolated to any other AD-suppressive agent encompassed by the present invention. Yet, administration of the synergist alone has produced minimal or no effect in attenuating either the specific immune response of a mammal to a particular autoantigen, or the ability of the mammal to mount an immune response to an exoantigen (i.e., a true foreign antigenic substance). Use of the synergist of the present invention thus affords this important two-fold advantage among others: enhancement of the suppression of the specific immune response to an autoantigen and no substantial effect on the ability of the subject so treated to mount an immune response against invading pathogens.

Various model systems have been developed for studying autoimmune diseases. Experimental allergic encephalomyelitis (EAE) has been studied in mice and other mammalian species as a model for Multiple Sclerosis (MS). The disease is induced by parenteral administration of MBP and an adjuvant (such as Freund's complete adjuvant). This treatment induces both a monophasic and an exacerbating/remitting form of demyelinating disease (depending on the species and details of administration). The induced disease has the characteristics of MS. Parenteral administration of Mycobacterium tuberculosis with Freund's complete adjuvant oil into the dorsal root tail of susceptible mammals induces a disease with the characteristics of human rheumatoid arthritis. In addition, the administration to Lewis rats of S-antigen and an adjuvant induces autoimmune uveoretinitis whereas diabetes develops spontaneously in the NOD mouse and the BB rat. Various ones of these model systems may be employed to demonstrate the efficacy and improved treatment provided by the present invention.

Non-limiting examples of autoimmune diseases which are cell-mediated include multiple sclerosis, rheumatoid arthritis, autoimmune uveoretinitis, diabetes (especially Juvenile Onset diabetes) and autoimmune thyroiditis. A non-limiting list of disease models and the specific autoantigens effective in the

treatment of these diseases when administered in an oral or enteral form are set forth in Table 1 below.

TABLE 1

	<u>Disease Model</u>	<u>Specific Autoantigen</u>
5	Multiple Sclerosis	MBP
	Rheumatoid Arthritis	Collagen
	Autoimmune Thyroiditis	Thyroglobulin
	Autoimmune uveoretinitis	S-antigen
	Diabetes	Islet cell extract
10	Chronic Active Hepatitis	Liver extract
	Adrenalitis	Adrenal gland extract
	Polymyositis	Muscle extract

For any autoimmune disease, tissue extracts can be used as well as the specific antigens described above.

15 Other autoimmune diseases and their specific autoantigens and/or target tissues are disclosed in Schwartz, R.S. et al. in Fundamental Immunology, Second Edition, Paul W.E., Ed., pg 819-859, Raven Press, NY, 1989.

20 Autoantigens for use in the present invention can be isolated from the tissue which is the target for the particular autoimmune disease. For example, myelin basic protein (MBP) for use in treating MS can be isolated and purified from mammals using the method of Diebler et al. (infra) as shown in Example 1 below.

25 When treating a disease having the symptoms of rheumatoid arthritis, collagen can be isolated and purified by the method of Trentham et al., J. Exp. Med. 146: 857, 1977.

30 For treating autoimmune uveoretinitis, purified S-antigen can be obtained as described in copending U.S. Patent Application Serial No. 379,778.

Fragments and analogs of autoantigens for use in the present invention can be synthesized using solid phase synthesis techniques well-known in the art such as those of Merrifield, R.B. (Fed. Proc. Am. Soc. Ex. Biol. 21: 412, 1962 and J. Am. Chem. Soc. 85: 2149, 1963) and Mitchel, A.R. et al., (J. Am. Chem. Soc. 98: 7357, 1976). as well as Tam, J. et al., (J. Am. Chem. Soc. 105: 6442, 1983) Analogs can be constructed

by identifying an equivalent amino acid sequence and using the peptide synthesis techniques disclosed above.

5 Analogues can be provided using for example the known amino acid sequence of GP-MBP as disclosed in G. Hashim, in Myelin: Chemistry and Biology Alan R. lisa, N.Y., 1980 using techniques described above and in Eyler, E.H., in Advances in Experimental Medicine and Biology 98: 259-281, 1978. For example, a peptide having a sequence corresponding to guinea pig MBP (GP-MBP) amino acid residues 72-85 as disclosed in
10 Hashim (supra) can be chemically synthesized using the above-described techniques with an amino acid substitution at the terminal asparagine position to glutamine. The peptide can be tested for disease-suppressive activity when administered in oral or enteral form using the techniques described in Example
15 2 below.

Disease-suppressive analogues and fragments can also be obtained using recombinant DNA techniques well-known in the art.

20 In another aspect, the present invention also provides pharmaceutical formulations for treating mammals suffering from autoimmune diseases comprising an amount of a synergist (as described below) effective to augment the autoimmune disease-suppressive effect of the AD-suppressive agent and a pharmaceutically acceptable carrier or diluent. The formulation
25 may also comprise an AD-suppressive agent specific for the autoimmune disease in an amount effective (singly or in conjunction with the amount of the synergist) to treat or prevent the clinical symptoms of specific autoimmune disease. It will be understood that any statistically significant
30 attenuation of even one symptom of an autoimmune disease pursuant to the treatment of the present invention is within the scope of the invention.

Each formulation according to the present invention may additionally comprise inert constituents including pharmaceutically acceptable carriers, diluents, fillers solubilizing or
35 emulsifying agents, and salts as is well-known in the art. For example, tablets may be formulated in accordance with conven-

tional procedures employing solid carriers well-known in the art. Capsules employed in the present invention may be made from any pharmaceutically acceptable material such as gelatin or cellulose derivatives. Sustained release oral delivery systems and/or enteric coatings for orally administered dosage forms are also contemplated such as those described in U.S. Patent No. 4,704,295 issued November 3, 1987, U.S. Patent No. 4,556,552 issued December 3, 1985, U.S. Patent No. 4,309,404 issued January 5, 1982, and U.S. Patent No. 4,309,406 issued January 5, 1982.

Examples of solid carriers include starch, sugar, bentonite, silica and other commonly used carriers. Further non-limiting examples of carriers and diluents which may be used in the formulations of the present invention include saline, syrup, dextrose and water.

It will be appreciated that the unit content of active ingredient or ingredients contained in an individual dose of each dosage form need not in itself constitute an effective amount since the necessary effective amount can be reached by administration of a plurality of dosage units (such as capsules or tablets or combinations thereof).

The route of administration of the synergists of the present invention is preferably oral or enteral. Preferred oral or enteral pharmaceutical formulations may comprise for example, a pill or capsule containing between about 1 mg and about 300 mg of one or more synergists of the present invention with or without an effective amount of the AD-suppressive agent specific for the autoimmune disease.

In general, the autoantigen, fragment, or analog is introduced to a mammal, orally or enterally, in an amount of between about 15 micrograms per kg body weight of said mammal and about 15 mg per kg body weight of said mammal per day, and may be administered in single dose form or multiple dose form. Preferably the autoantigen, fragment, or analog is administered in an amount between about 300 micrograms and about 12 mg per kg body weight of said mammal per day.

The synergist may be administered to a mammal preferably orally or enterally in an amount broadly ranging between about 15 mg and about 15 mg per kg body weight of said mammal per day and preferably ranging between about 300 mg and about 12 mg per kg body weight per day and also may be administered in a single or multiple dose form. The synergist may be orally or enterally administered before (within 24 hours and preferably within 1 hour) or substantially simultaneously with or after (within 24 hours after) the administration of the autoantigen or biologically active fragment or analog of said autoantigen specific for said disease. As will be understood by one skilled in the art, the exact dosage and frequency of administration is a function of the activity of the AD-suppressive agent and the synergist as well as the age, sex, weight, and physical condition of the subject to be treated, and the concurrent administration or absence of other treatments. Consequently, adjustment of the dosages used and administration schedules must be determined based on these factors and may need to be determined experimentally. Such determination, however, requires no more than routine experimentation, as disclosed by Higgins and Weiner for EAE in J. Immunol 140: 440-445, 1988.

As shown in Example 8 below, subcutaneous administration of IL-1 enhanced the AD-suppressive effect of orally administered MBP on EAE. This is a most surprising finding in that IL-1 has been found to have an immunostimulatory effect on helper T-cells, the mediators of EAE and thus would be expected to exacerbate disease symptoms. Therefore, although oral or enteral administration of the synergist (which induces IL-1 production and/or secretion) is preferred, parenteral administration of IL-1, and preferably subcutaneously, to a mammal at a concentration ranging between about 0.2 mg and about 60 mg per kg of body weight of said mammals, combined with oral or enteral administration of an AD-suppressive agent (in dosages described above) is also within the scope of the present invention. IL-1 for use in the present invention is commercially available from numerous commercial sources such as

Boehringer Mannheim (Indianapolis, IN), Amgen Biologicals (Thousand Oaks, CA) and ICN Biochemicals (Cleveland, OH).

Where the AD-suppressive agent and synergist are introduced orally, they may be mixed with other food forms and consumed in solid, semi-solid, suspension, or emulsion form; they also may be mixed with pharmaceutically acceptable carriers, flavor enhancers and the like.

Where the AD-suppressive agent and synergist are administered enterally, they may be introduced in solid, semi-solid, suspension or emulsion form and may be compounded with any of a host of well-known, pharmaceutically acceptable carriers, including water, suspending agents, and emulsifying agents.

The AD-suppressive agent and the synergist may be administered together in the same dosage form, co-administered by ingesting separate dosage forms, or administered sequentially in separate dosage forms.

Examples 2-8 illustrate the use of a synergist in accordance with the present invention in conjunction with an AD-suppressive agent and the specific tolerance-enhancing effect of such joint use.

According to Examples 2-8, feeding bacterial lipopolysaccharide (LPS) or the Lipid A moiety of LPS (LPS is composed of a Lipid A moiety and a polysaccharide moiety) used as the synergist enhanced the protective effect of feeding MBP on EAE in Lewis rats. The induction of disease, the disease severity and the Disease Index were all reduced as a result of administration of an AD-suppressive agent with a synergist as opposed to the oral administration of an AD-suppressive agent alone. The synergists of the present invention were also effective in suppressing disease symptoms when administered with the autoantigen during the active disease phase as shown in Example 6 using a chronic EAE model in guinea pigs.

The feeding of MBP-plus-LPS or MBP-plus-Lipid A also led to a significant decrease in the delayed type hypersensitivity (DTH) reactions of the treated animals. EAE and DTH are both believed to be CD4+ T-cell-mediated phenomena.

Consequently, the decrease in DTH corroborates the effect of the synergists of the present invention. In both the EAE and DTH systems Lipid A or LPS had little or no effect when added alone in the absence of the autoantigen, MBP. Therefore, the tolerance enhancing effect of the synergist is not nonspecific.

It is anticipated that the synergists of the present invention will augment or enhance the suppressive effect obtained by the oral or enteral administration of AD-suppressive agents in the treatment of other autoimmune diseases such as arthritis, diabetes, as well as the autoimmune diseases such as those exemplified above in Table 1.

In addition, as shown in Example 7 below, orally-administered MBP plus LPS caused a decreased expression of Class II histocompatibility molecules relative to Class I histocompatibility molecules in MBP-fed rats on gut epithelial cells isolated from treated animals. Without wishing to be bound by theory, it is hypothesized that by differentially decreasing Class II-expression, LPS may cause a reduction in antigen presentation to CD4+ T-cells (helper T-cells), which are inducer cells. Since oral MBP tolerance induction is believed to operate via a different mechanism, i.e. induction of suppressor T-cells, a synergistic effect is observed when LPS and MBP are administered together.

The present invention is further described below in specific Examples which are intended to further describe the invention without limiting its scope.

EXAMPLE 1:

Autoantigens used in practicing the method of the present invention were obtained using the method and techniques set forth below.

GP-MBP was purified by the method of Diebler, G.E. et al. (Prep. Biochem. 2: 139, 1972) from guinea pig brain tissue and was obtained from Pel Freeze (Rogers, Arkansas). Briefly, central nervous system tissue was isolated and homogenized in a chloroform-methanol solution, extracted in acetone, filtered and resuspended in the same solution. The solution was extracted in acetone, filtered, resuspended in water, adjusted

to pH 3.0 and incubated for 1 hour. The solution was then centrifuged and extracted with 8 M urea, CM-5 added, the pH adjusted to 11, and the solution filtered and resuspended twice in urea. The solution was then filtered again, resuspended twice in water, filtered, resuspended in 0.121 N HCl, filtered, dialyzed against 10 volumes of distilled water and lyophilized before use.

EXAMPLE 2: **Feeding LPS Enhances the Protective Effect of Feeding MBP on EAE**

A series of experiments were performed in the Lewis rat in which Lewis rats age 6-8 weeks (Harlan-Sprague Dawley, Indianapolis, IN) were fed MBP (1 mg in PBS) or LPS (1 mg in PBS) alone or a combination of MBP + LPS (1 mg and 1 mg) by gastric intubation using an 18-gauge stainless steel animal feeding needle (Thomas Scientific, Swedesboro, NJ). The rats were fed five times (total dose 5 mg) before immunization. LPS (Sigma Chemical Co. St. Louis, MO) was from E. coli strain 0127:B8. The rats were then immunized in each foot pad with 25 micrograms MBP in an equal volume of complete Freund's adjuvant (CFA) containing 4 micrograms of Mycobacterium tuberculosis (Difco, Detroit, MI) 2 days after the last feeding and observed for EAE disease over the following 2-3 weeks. Disease is manifested by paralysis. The seventy scoring system was: 0 = normal; 1 = limp tail; 2 = hind leg paralysis; 3 = incontinence; 4 = tetraplegia; and 5 = moribund. EAE severity varied between 2 and 5 depending upon the experiment. Disease severity was also scored as "Disease Index". The Disease Index was calculated for each animal and consisted of duration of disease (in days) times the highest disease score. Animals were scored clinically in a blinded fashion. The results are set forth in Table 2 below:

TABLE 2

Oral LPS Enhances Orally Induced
Suppression of EAE by MBP

	<u>TREATMENT</u>	<u>INCIDENCE</u>	<u>DURATION</u> (Days)	<u>MEAN MAXIMAL</u> <u>SEVERITY</u>	<u>DISEASE</u> <u>INDEX</u>
5	None (Control)	22/22	6.85 \pm 0.39	2.98 \pm 0.38	18.60 \pm 2.03
10	LPS fed	20/24	4.58 \pm 0.56 ^b	1.89 \pm 0.25 ^a	11.15 \pm 1.84 ^a
	MBP fed	21/29 ^b	3.24 \pm 0.50 ^c	1.17 \pm 0.18 ^c	5.83 \pm 1.18 ^c
15	MBP+LPS fed	12/29 ^b (p<0.05) ^d	1.97 \pm 0.53 ^c (N.S.) ^d	0.53 \pm 0.13 ^c (p<0.05) ^d	2.72 \pm 0.79 ^c (p<0.05) ^a

a p < 0.06 vs. control

20 b p < 0.01 vs. control

c p < 0.001 vs. control

d p values comparing LPS + MBP vs. MBP

[p values were calculated by student t-test except for the incidence, where chi-square was used.]

25 The experiments described above demonstrated a marked augmentation of protection against the disease in animals that were fed both LPS and MBP as compared to those fed MBP alone. MBP + LPS lowered the incidence of disease (12/29 animals versus 22/22 for controls and 12/29 animals for MBP-fed), the duration of disease, the mean maximal severity and the DI. Of note is that feeding LPS alone had a lesser effect on the animals' susceptibility to EAE than either MBP or MBP + LPS. The results clearly demonstrate a superadditive or synergistic enhancement of disease suppression due to the joint use of an AD-suppressive agent (MBP) and a synergist (LPS).

EXAMPLE 3: The Lipid A Moiety of LPS is Equally Effective

40 LPS is a structure that contains both a lipid moiety plus a polysaccharide chain (Chiller, J. M. et al., Proc. Nat. Acad. Sci USA 70: 2129-2133, 1973). In experiments performed it was found that Lipid A functioned equally well or better than LPS as a synergist in protecting against EAE. Rats (9-10 per group) were fed with Lipid A (75 micrograms per rat, from Salmonella minnesota Re 595, obtained from Sigma Chemical Co.), following the same protocol used for LPS feeding in Example 2

above. EAE was induced as described in Example 2 above. The results are set forth in Table 3 below.

TABLE 3

Lipid A Is The Active Part of the LPS Molecule

<u>Treatment</u>	<u>Incidence</u>	<u>Duration (Days)</u>	<u>Maximal Grade</u>	<u>Disease Index</u>
None (Control)	10/10	7.70 \pm 0.26	3.1 \pm 0.10	26.20 \pm 1.94
Lipid A	9/9	7.56 \pm 0.34 ^a	3.40 \pm 0.24 ^a	24.33 \pm 1.76 ^a
MBP	10/10	6.40 \pm 0.45 ^b	1.9 \pm 0.23 ^c	11.79 \pm 1.94 ^d
MBP+Lipid A	3/9	3.44 \pm 1.16 ^c (p < 0.05) ^e	0.03 \pm 0.29 ^d (p < 0.01) ^e	6.50 \pm 2.1 ^d (p < 0.05) ^e

a p not significant

b p < 0.06

c p < 0.01

d p < 0.001

e p values of MBP vs. MBP+Lipid A

As can be seen from the results presented in Table 3 above, Lipid A, at a dose of 75 micrograms administered orally with MBP, significantly enhanced protection against EAE with a decrease in the Disease Index (DI) from 12.12 for controls and 11.6 for MBP fed rats to 0.06 for rats fed MBP+Lipid A (Table 3). In addition, MBP+Lipid A fed rats had a lower incidence of disease (33% versus 100% for all other groups), a shorter duration of disease and a lower maximal grade when compared to control, MBP-fed or Lipid A-fed animals.

EXAMPLE 4: Delayed Type Hypersensitivity Reactions

The experiments of Examples 2 and 3 outlined above monitor disease expression measured by paralysis. A series of experiments were performed to determine whether other immune measurements would also demonstrate a superadditive effect as a result of use of the synergist. Since EAE is believed to be a CD4+ T-cell mediated disease, and this cell is the primary cell involved in Delayed Type Hypersensitivity (DTH) reactions, DTH responses in animals treated with LPS were measured to determine whether suppression could be observed. These DTH reactions were done in a standard fashion by measuring ear swelling

48 hours following the injection of 50 microliters of the autoantigen (MBP, 200 micrograms/ml) into the pinna of a rats ear that were not exposed to MBP, sensitized with MBP 200 micrograms/ml, and/or fed 1 mg MBP and/or LPS (1 mg) prior to challenge. As a control, 50 microliters of Mycobacterium tuberculosis (Mt) at a concentration of 200 micrograms/ml was administered to the MBP sensitized rats. The measurements were performed as in J. Immunol. 125:483,1980 and are expressed as the mean change in ear swelling in inches $\times 10^{-2}$. The results are shown in Figures 1 and 2. In Figures 1 and 2, the ordinate depicts the mean difference in thickness of the rats' ears before and 48 hours after MBP injection. Figure 1 depicts the DTH results in rats fed LPS and Figure 2 depicts the DTH results in rats fed Lipid A.

A marked and significant decrease (45%) in DTH responses was found in animals fed a combination of either MBP and Lipid A or LPS. Neither LPS nor Lipid A alone had any effect in inhibiting ear-swelling (Figures 1 and 2, respectively). The suppression of DTH following MBP and LPS or Lipid A administration was antigen specific, as DTH responses to Mt were unaffected.

EXAMPLE 5: Route of Administration Influences
 Effect of Synergist

In order to study the mechanism of action of the synergists of the present invention, an experiment was performed according to the protocol as set forth in Example 2 above in which the synergist (LPS) was administered (at a dose of 1 milligram) at the same time as 1 milligram of MBP administered 5 times as in Example 2 above either orally ("PO") or subcutaneously ("SQ"). Controls were simply challenged with injected MBP. The results are set forth in Table 4 below.

TABLE 4

LPS Enhances Oral Tolerance Better When Given Orally
Than When Given By Another Route

5

	<u>TREATMENT</u>	<u>INCIDENCE</u>	<u>DURATION</u>	<u>MEAN MAXIMAL SEVERITY</u>	<u>DISEASE INDEX</u>
	None (Control)	5/5	7.6 ± 0.51	3.2 ± 0.37	24.8 ± 3.78
10	MBP fed	5/5	5.4 ± 0.40 ^a	1.8 ± 0.37 ^b	10.0 ± 2.45 ^b
	MBP+LPS PO	2/5	1.8 ± 0.11 ^b (p<0.05) ^d	0.8 ± 0.49 ^b (p<0.05) ^d	3.6 ± 2.23 ^a (p<0.05) ^d
15	MBP+LPS SQ	4/4	6.0 ± 0.71 ^c	2.7 ± 0.25 ^c	15.52 ± 3.28 ^c
	LPS SQ	4/4	5.0 ± 0.47 ^c	4.75 ± 0.25 ^b	22.75 ± 5.4 ^c

20

a p < 0.01

b p < 0.05

c NS

25

d p values of MBP+LPS PO vs. MBP+LPS SQ

30

Oral LPS decreased the DI from 23.04 in controls and 8.0 in MBP fed rats to 0.26 in MBP+LPS fed rats. Subcutaneous LPS, on the other hand, abrogated the tolerance following MBP feeding, the DI was 16.5 compared to 8.0 for MBP alone (Table 4). DTH responses to MBP were measured in these rats, and the results correlate with those obtained for clinical disease. MBP with oral LPS suppressed the DTH response more than MBP alone (data not shown). In the rats treated with subcutaneous injection of LPS and oral MBP, mean ear swelling was not significantly different from those of control rats (data not shown).

35

EXAMPLE 6: Administering the Synergist Post-Induction of EAE

40

In order to examine the effects of the synergists of the present invention on an active, ongoing autoimmune disease, a chronic EAE model was studied. Strain 13 guinea pigs (Associated Rabbit Industries, East Bridgewater, MA) were sensitized with a 1:1 mixture of guinea pig myelin (purchased from Pel-Freez, Rogers, AR) and sterile physiological saline with an

equal volume of Incomplete Freund's adjuvant containing 4 micrograms of heat killed Mycobacterium tuberculosis (Difco, Detroit, MI). 0.4 ml was injected into several sites in the nuchal area.

5 Animals were fed 10 mg of a bovine myelin preparation (Biopure, Boston, MA) with and without 150 micrograms of Lipid A as in Example 2 above, 3 times a week following the first attack of EAE on day 32 post-immunization. The animals were evaluated every other day for incidence of disease in a blinded
10 fashion for 120 days. The disease was characterized by hind limb paralysis and incontinence. The disease was graded according to the clinical severity as follows: 0 = no abnormality; 1 = weakness, limpness, decreased activity; 2 = mild paraparesis; 3 = moderate paraparesis; 4 = severe paraparesis;
15 5 = death. The clinical severity score was generated by taking the actual score times the numbers of observations divided by the total potential maximal at-risk score (the maximal potential at-risk score assumes that every animal is a 4 in clinical severity) and multiplying times 100. The results are shown in
20 Figure 3.

Figure 3C shows the control animals. The clinical scores for these animals (in Figures 3A-3C, each bar graph represents the results of one individual animal) ranged between 61 and 87 (Figure 3C, average 73). Oral bovine myelin ad-
25 ministration reduced the clinical scores to 19-72 (Figure 3B, average 53) whereas oral administration of bovine myelin plus Lipid A further reduced the clinical scores to 19-26 (Figure 3A, average 22), demonstrating the efficacy of orally administering synergists with AD-suppressive agents to treat an
30 ongoing autoimmune disease.

EXAMPLE 7: Gut Epithelial Cells from Animals fed MBP plus LPS Express Differential Amounts of Class I and Class II Molecules

35 In these experiments gut epithelial cells were isolated from 6- to 8-week old female Lewis rats that had been fed either MBP or MBP plus LPS. Gut mucosal epithelial cells were obtained from normal Lewis rats, as described in Bland, P.W., et al. Immunol., 58:1, 1986. The small intestine, comprising

duodenum and proximal jejunum was opened longitudinally, washed extensively in HBSS (Hanks balanced salt solution) and incubated at room temperature for 5 minutes in HBSS containing 0.5 mM EDTA. The villous epithelium and mucus were removed by rubbing the mucosa with a rubber policeman. The resulting clumps were then disrupted into a single cell suspension using a Pasteur pipette. Cells were then washed in RPMI 1640 medium containing 10% FCS (fetal calf serum) and, counted in a hemocytometer. Cells were then stained with monoclonal antibodies to Class I or Class II determinants and enumerated on a Fluorescence Activated Cell Sorter (FACS) as described in Santos, L.M. B. et al., Cellular Immunol (In press).

The amounts fed were 1 microgram of MBP or 1 microgram of MBP together with 1 microgram of LPS. The following percentages of cells expressed Class II-antigen: 53.8% of epithelial cells from control rats; 65.9% from MBP fed rat; and 16.9% from MBP plus LPS fed rats. The following percentages of cells expressed Class I-antigen: control rats 86.7%; MBP-fed rats 98.4%; MBP plus LPS fed rats 61%. These results demonstrate differential effects on Class II histocompatibility antigen expression of epithelial cells from MBP plus LPS fed rats. These results suggest that one of the possible mechanisms by which LPS acts involves differentially decreasing Class II expression (relative to Class I expression) on epithelial cells. Thus, these cells do not preferentially present antigen to CD4+ cells which are inducer (or helper) cells as opposed to suppressor cells.

EXAMPLE 8:

In order to examine the mechanism of action of the synergists of the present invention, Lewis rats (5 per group) in which EAE had be induced as in Example 2, above were treated orally with 1 mg of GP-MBP alone, GP-MBP (1 mg) plus LPS (1 mg) (oral), IL-1 (200 micrograms, intraperitoneally administered) or fed GP-MBP (1 mg) + injected with IL-1 (200 micrograms intraperitoneally administered) 2-3 days after last feeding and the disease index measured. The results are set forth in Table 5 below:

TABLE 5

	<u>Treatment</u>	<u>Disease Index</u>
	None (Control)	23
5	GP-MBP	19
	GP-MBP + LPS	17
	IL-1	28
	GP-MBP + IL-1	4

10 The results presented in Table 5 above show that oral administration of 1 milligram of GP-MBP reduced the Disease Index from 23 to 19 and that inclusion of LPS with the GP-MBP further lowered the Disease Index to 17. Injection of 200 micrograms of IL-1 alone (intrapertoneally) increased the Disease Index in the treated rats to 28. However, oral ad-
15 ministration of 1 milligram of GP-MBP plus injection of 200 micrograms of IL-1 reduced the Disease Index to 4.

Therefore, the above results show that parenterally-administered IL-1 was able to synergize with oral MBP administration.

WHAT IS CLAIMED IS

- 1 1. A method for treating or preventing an autoimmune
2 disease (AD) presenting the symptoms of multiple sclerosis in a
3 mammal in need of such treatment comprising:
4 (a) orally or enterally administering to said
5 mammal at least one AD-suppressive agent selected from the
6 group consisting of (i) autoantigens specific for said autoim-
7 mune disease, (ii) AD-suppressive fragments of said
8 autoantigens, and (iii) AD-suppressive analogs of said
9 autoantigens or fragments; and
10 (b) at least one compound having the property of
11 enhancing the AD-suppressive activity of said agent,
12 the amounts of (a) and (b) in combination
13 effective to suppress said symptoms.
- 1 2. The method of claim 1 wherein said autoantigen is
2 myelin basic protein.
- 1 3. The method of claim 1 wherein said compound is a
2 bacterial lipopolysaccharide.
- 1 4. The method of claim 1 wherein said compound is
2 Lipid A.
- 1 5. The method of claim 1 wherein said compound is
2 administered orally or enterally.
- 1 6. The method of claim 5 comprising simultaneous ad-
2 ministration of said agent and said compound.
- 1 7. The method of claim 1 comprising administering to
2 said mammal an amount of said agent individually effective to
3 suppress or prevent the clinical symptoms of said disease.
- 1 8. The method of claim 1 comprising administering to
2 said mammal an amount of said agent and an amount of said

3 compound said amounts being effective in combination to
4 suppress said symptoms.

1 9. A method for treating or preventing a T-cell
2 mediated or T-cell dependent autoimmune disease (AD) the method
3 comprising:

4 (a) orally or enterally administering to a mammal
5 in need of such treatment at least one AD-suppressive agent
6 selected from the group consisting of (i) autoantigens specific
7 for said disease; (ii) AD-suppressive fragments of said
8 autoantigens; and (iii) AD-suppressive analogs of said
9 autoantigens or said fragments; and

10 (b) also administering to said mammal at least one
11 compound having the property of enhancing the AD-suppressive
12 activity of said agent;

13 in amounts effective to suppress said disease.

1 10. The method of claim 9 wherein said compound is a
2 bacterial lipopolysaccharide.

1 11. The method of claim 9 wherein said compound is
2 Lipid A.

1 12. The method of claim 9 comprising administering
2 said compound before administration of said agent.

1 13. The method of claim 9 comprising administering
2 said compound substantially simultaneously with said agent.

1 14. The method of claim 9 comprising administering
2 said compound orally or enterally.

1 15. The method of claim 9 comprising administering
2 said compound parenterally.

1 16. A method for treating or preventing a T-cell

2 mediated or T-cell dependent autoimmune disease (AD) the method
3 comprising:

4 (a) orally or enterally administering to a mammal
5 in need of such treatment at least one AD-suppressive agent
6 selected from the group consisting of (i) autoantigens specific
7 for said disease; (ii) AD-suppressive fragments of said
8 autoantigens; and (iii) AD-suppressive analogs of said
9 autoantigens or said fragments; and

10 (b) also administering to said mammal at least one
11 compound having the property of enhancing the AD-suppressive
12 activity of said agent;

13 in amounts effective to differentially decrease
14 the expression of Class II histocompatibility antigens relative
15 to Class I histocompatibility antigens by gut epithelial cells
16 of said mammal.

1 17. A pharmaceutical formulation for treating or
2 preventing the clinical symptoms of an autoimmune disease
3 comprising an effective amount of

4 (a) an AD-suppressive agent selected from the
5 group consisting of (i) autoantigens specific for said autoim-
6 mune disease, (ii) AD-suppressive fragments of said
7 autoantigens, and (iii) AD-suppressive analogs of said
8 autoantigens or fragments;

9 (b) at least one compound having the property of
10 enhancing the AD-suppressive activity of said agent; and

11 (c) a physiologically acceptable carrier or
12 diluent.

1 18. A pharmaceutical formulation comprising an oral
2 dosage form containing an effective amount for enhancing the
3 AD-suppressive activity of an AD-suppressive agent comprising
4 at least one compound having the property of enhancing the AD-
5 suppressive activity of said agent and a physiologically accep-
6 table carrier.

1 / 3

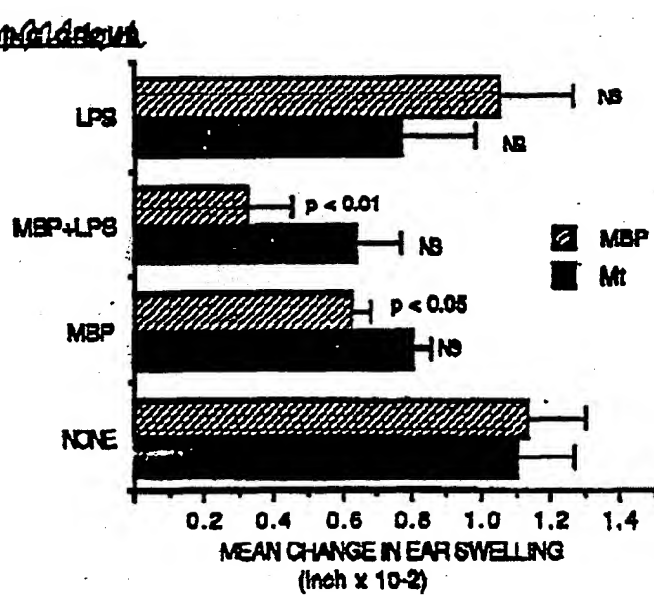
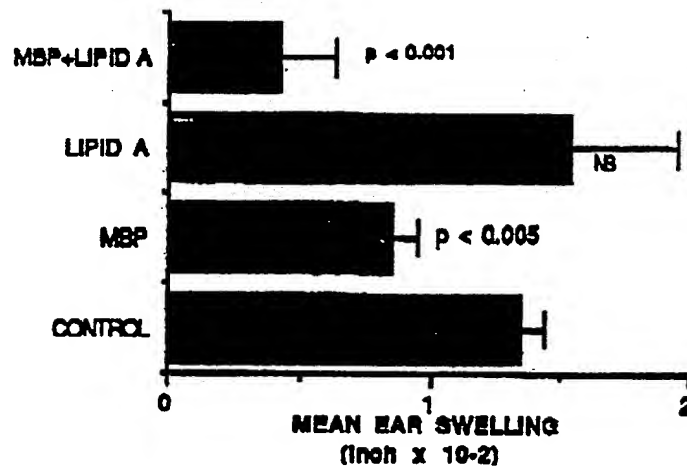
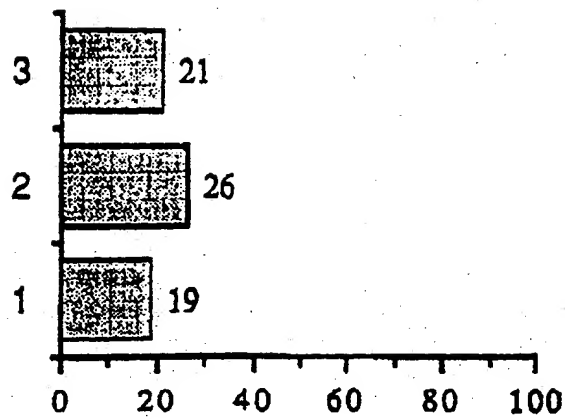


Figure 1

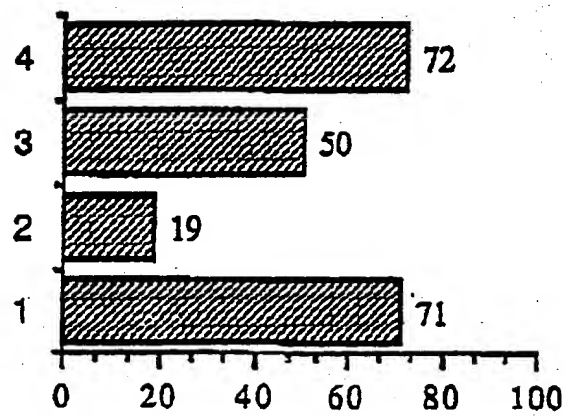
2 / 3

FEEDING GROUP*Figure 2*

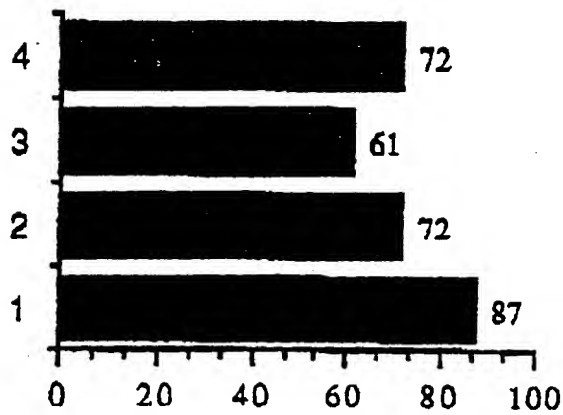
3 / 3



A



B



C

clinical score

Figure 3

INTERNATIONAL SEARCH REPORT

International Application No. **PCT/US91/01466**

I. CLASSIFICATION & SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶ According to International Patent Classification (IPC) or to both National Classification and IPC IPC(5): A61K 37/20, 39/04 U.S. CL: 424/85.1, 92, 85.8														
II. FIELDS SEARCHED <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Minimum Documentation Searched ⁷</div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 20%; text-align: left; border-bottom: 1px solid black;">Classification System</th> <th style="text-align: left; border-bottom: 1px solid black;">Classification Symbols</th> </tr> <tr> <td style="border: 1px solid black; padding: 5px;">U.S.</td> <td style="border: 1px solid black; padding: 5px;">424/85.1, 85.8, 92</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸</div> <p>Databases: Dialog (files 155, 154, 72, 172, 399, 357), USPTO Automated Patent System (File USPAT, 1971-1991)</p>			Classification System	Classification Symbols	U.S.	424/85.1, 85.8, 92								
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III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹ <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 10%; text-align: left; border-bottom: 1px solid black;">Category [*]</th> <th style="text-align: left; border-bottom: 1px solid black;">Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²</th> <th style="width: 15%; text-align: left; border-bottom: 1px solid black;">Relevant to Claim No. ¹³</th> </tr> <tr> <td style="border: 1px solid black; vertical-align: top; padding: 5px;"> <div style="text-align: center;">X Y</div> </td> <td style="border: 1px solid black; vertical-align: top; padding: 5px;"> The Journal of Immunology, Vol. 140, No. 2, issued 01 January 1988, Higgins et al., "Suppression of Experimental Autoimmune Encephalomyelitis By Oral Administration of Myelin Basic Protein and Its Fragments", pages 440-445, see entire article. </td> <td style="border: 1px solid black; vertical-align: top; padding: 5px;"> <div style="text-align: center;">1-8 9-15</div> </td> </tr> <tr> <td style="border: 1px solid black; vertical-align: top; padding: 5px;"> <div style="text-align: center;">X Y</div> </td> <td style="border: 1px solid black; vertical-align: top; padding: 5px;"> Journal of Experimental Medicine, Vol. 169, issued May 1989, Clayton et al., "Peptide-specific Prevention of Experimental Allergic Encephalomyelitis", pages 1681-1691, see entire article. </td> <td style="border: 1px solid black; vertical-align: top; padding: 5px;"> <div style="text-align: center;">1-8 9-15</div> </td> </tr> <tr> <td style="border: 1px solid black; vertical-align: top; padding: 5px;"> <div style="text-align: center;">X Y</div> </td> <td style="border: 1px solid black; vertical-align: top; padding: 5px;"> Journal of Immunology, Vol. 126, No. 4, issued April 1981, Lando et al., "The Immunologic Response in Mice Unresponsive to Experimental Allergic Encephalomyelitis" page 1526-1528, see abstract. </td> <td style="border: 1px solid black; vertical-align: top; padding: 5px;"> <div style="text-align: center;">1-8 9-15</div> </td> </tr> </table>			Category [*]	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³	<div style="text-align: center;">X Y</div>	The Journal of Immunology, Vol. 140, No. 2, issued 01 January 1988, Higgins et al., "Suppression of Experimental Autoimmune Encephalomyelitis By Oral Administration of Myelin Basic Protein and Its Fragments", pages 440-445, see entire article.	<div style="text-align: center;">1-8 9-15</div>	<div style="text-align: center;">X Y</div>	Journal of Experimental Medicine, Vol. 169, issued May 1989, Clayton et al., "Peptide-specific Prevention of Experimental Allergic Encephalomyelitis", pages 1681-1691, see entire article.	<div style="text-align: center;">1-8 9-15</div>	<div style="text-align: center;">X Y</div>	Journal of Immunology, Vol. 126, No. 4, issued April 1981, Lando et al., "The Immunologic Response in Mice Unresponsive to Experimental Allergic Encephalomyelitis" page 1526-1528, see abstract.	<div style="text-align: center;">1-8 9-15</div>
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<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>[*] Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>														
IV. CERTIFICATION <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;"> Date of the Actual Completion of the International Search 21 May 1991 </td> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;"> Date of Mailing of this International Search Report <div style="text-align: center; font-size: 1.2em; font-weight: bold;">14 JUN 1991</div> </td> </tr> <tr> <td style="border-bottom: 1px solid black; padding: 5px;"> International Searching Authority ISA/US </td> <td style="border-bottom: 1px solid black; padding: 5px;"> Signature of Authorized Officer <div style="text-align: center;"> Lynette F. Smith </div> </td> </tr> </table>			Date of the Actual Completion of the International Search 21 May 1991	Date of Mailing of this International Search Report <div style="text-align: center; font-size: 1.2em; font-weight: bold;">14 JUN 1991</div>	International Searching Authority ISA/US	Signature of Authorized Officer <div style="text-align: center;"> Lynette F. Smith </div>								
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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
<u>X</u> Y	Science, Vol. 244, issued 19 May 1989, Lohse et al., "Control of Experimental Autoimmune Encephalomyelitis by T cells Responding to Activated T cells", pages 820-822, see entire article.	16-18
<u>X</u> Y	Nature, Vol. 341, issued 12 October 1989, Vanderbark et al., "Immunization with A Synthetic T Cell Receptor V-region Peptide Protects Against Experimental Autoimmune Encephalomyelitis", pages 541-544, see entire article.	16-18
X	US, A, 4,695,459 (Steinman et al.) 22 September 1987, see entire document.	16-18
<u>X</u> Y	Journal of Autoimmunity, Vol. 1, No. 5, issued October 1988, Jonker et al., "Successful Treatment of EAE in Rhesus Monkeys with MHC class II specific Monoclonal Antibodies" pages 399-414, see Abstract. No. 89322538.	16-18